AD_____

Award Number: W81XWH-05-1-0226

TITLE: Prognostic Significance of Telomere Attrition in Ductal Carcinoma in Situ of the Breast

PRINCIPAL INVESTIGATOR: Jeffrey K. Griffith, Ph.D.

CONTRACTING ORGANIZATION: University of New Mexico
Albuquerque, NM 87131

REPORT DATE: February 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-02-2006	Annual	31 Jan 2005 - 30 Jan 2006

4. TITLE AND SUBTITLE
Prognostic Significance of Telomere Attrition in Ductal Carcinoma in Situ of the Breast

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0226

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Jeffrey K. Griffith, Ph.D.

E-Mail: JKGriffith@salud.unm.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of New Mexico
Albuquerque, NM 87131

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: We are using an innovative, quantitative assay for telomere DNA content (TC) developed and characterized by the PI, to test the hypothesis that TC can be used to predict the likelihood of disease progression in women with DCIS. The first step in this investigation is to determine whether TC measured in bulk DCIS tumor tissue is comparable to that measured in tumor epithelial cells that have been stripped of stromal cells and connective tissue by laser-capture microscopy. We collaborated to initially measure TC in 10 pairs of microdissected and undissected tissue. In 7/10 instances, TC in the microdissected specimens was 72-112% of that in the undissected control (mean 85%). These initial results suggest that it will not be necessary to microdissect or otherwise fractionate DCIS specimens prior to TC analysis. We are currently confirming and extending these results in our own laboratory, with our own instrumentation. We also have obtained 97 samples of DCIS tissue from women with 4-9 years of continuing follow up for the subsequent retrospective study, have purified DNA, measured TC, and compared these data to TC measured in invasive breast tumors. We also are working with the New Mexico Tumor Registry to identify another 120 specimens of DCIS from women with longer follow up, including as many as possible who had recurrent disease. In summary, during the first year we have (i) demonstrated the ability measure TC in DCIS tissue microdissected by laser capture microscopy, (ii) derived preliminary evidence that it will not be necessary to microdissect DCIS tissue in the subsequent retrospective study, and (iii) begun to acquire and characterize the tissues that will comprise the retrospective study.

15. SUBJECT TERMS
Ductal carcinoma in situ (DCIS), breast cancer, telomeres, prognosis, genomic instability

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	9	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

Introduction

The widespread use of screening mammography has resulted in a ten fold increase in the incidence of ductal carcinoma in situ (DCIS) over the past twenty years, from 4800 cases in 1983 to more than 50,000 cases in the US in 2003 (1-3). This accounted for 18.3% of all newly diagnosed breast tumors, and 23% of newly diagnosed breast tumors in women 40-49 years of age in 2003 (2,3). However, the fraction of women with DCIS who eventually progress to invasive breast cancer is small (4-6). A Danish autopsy study found that 25% of women had *in situ* carcinomas, including DCIS, at their death, although the lifetime risk of developing breast cancer during the same period was only 1% (7). Similarly, only 32% of women whose DCIS was misdiagnosed as normal, and so did not receive treatment, went on to develop invasive carcinoma within 30 years of their biopsy (8,9). Less than 2% of women with DCIS die from breast cancer within ten years of diagnosis (10). Taken together, these results imply that up to two-thirds of women with DCIS would not progress to invasive cancer, even without treatment. Unfortunately, currently available prognostic markers are unable to discriminate between DCIS that will and will not progress, so many women receive aggressive treatments that may be unnecessary. Currently, 97.5% of women with DCIS in 1999 had some type of surgery, of which 28% had radical mastectomies (11). In the March, 2004 issue of the Journal of the National Cancer Institute, Baxter et al (11) wrote: "*The potential for preventing invasive breast cancer is important, yet the risk for over treatment is a clinically significant concern*". In an accompanying editorial, Dr. Morrow comments on barriers to developing meaningful therapeutic guidelines (12). She writes:

"*The first of these is our inability to identify which DCIS lesions will progress to invasive carcinoma, and in what time interval. Conventional prognostic factors, such as patient age and tumor grade, subtype, and size, provide information on the time course of local recurrence and the magnitude of risk reduction achieved with radiotherapy, but these factors do not identify those women who will have a disease recurrence with potentially life-threatening invasive cancer. Efforts to identify a molecular signature for DCIS lesions that will recur as invasive carcinoma are of enormous interest…*"

Using an innovative, quantitative assay for telomere DNA content (TC) developed and characterized by the PI (13-17), we have recently shown that TC in tumor tissue is associated with cancer-free survival in women with breast cancer (18). The purpose of this investigation is to determine whether TC can be used similarly to predict the likelihood of disease progression in women with DCIS.

Body

Tasks: The agreed upon tasks to be completed during the first year of the IDEA Award were:

Aim One: We will compare TC measured in bulk DCIS tumor tissue to TC measured in tumor epithelial cells that have been stripped of stromal cells and connective tissue by laser-capture microscopy.

Task 1	Months 1-6	Obtain 30 random archival specimens of DCIS.
Task 2	Months 2-12	Extract DNA from bulk DCIS samples and measure telomere DNA content (TC). Divide study group into thirds, based on TC.
Task 3	Months 4-12	Use laser capture microscopy to purify tumor epithelial cells from stromal and cells and connective tissues in the 10 samples comprising the middle third of the study group.
Task 4	Months 6-12	Extract DNA from purified epithelial cells and measure TC. Compare TC in bulk DCIS tissue to purified tumor epithelial cells.

Aim Two: The data from aim one will be used to guide the study design for the second aim, in which we will perform a retrospective study of the association between TC and time to disease recurrence in women with DCIS.

Task 5	Months 1-6	Design search parameters for NMTR database and identify 120 members of study group.
Task 6	Months 1-6	Establish data base of patient records
Task 7	Months 3-24	Obtain tissue blocks and cut new sections.
Task 8	Months 12-30	Extract DNA from 120 bulk specimens of DCIS or enriched epithelial cells, depending on the outcome of Aim One, and measure TC.

Progress Relative to Tasks:

Tasks 1 and 2 have been completed (Figure 1)

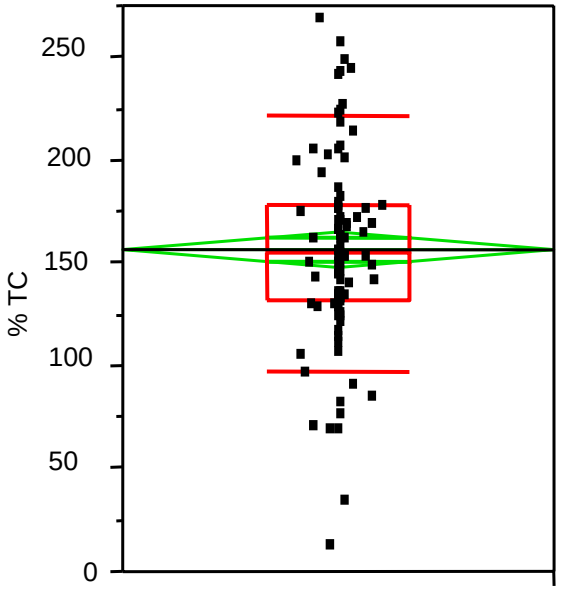

Figure 1. Distributions of Telomere DNA Contents (TC) in 97 Specimens of Bulk DCIS Tissues. TC is shown on the y-axis, and is expressed as a percentage of TC in placental DNA standard, measured in parallel. The 10th and 90th quantiles are shown as lines above and below the box. The line across the diamond represents the group mean. The height of the diamond represents the 95% confidence interval for the group.

Tasks 3 and 4 are partially complete. To minimize possible delays resulting from tissue acquisition, we initially collaborated with Dr. Colleen Fordyce to measure TC in 10 pairs of bulk DCIS tissue obtained from her laboratory (Figure 2). In 7/10 instances, TC in the microdissected specimens was 72-112% of that in the undissected control. The difference in TC in bulk and microdisected tissue was relatively constant (median 85%).We are currently confirming and extending these results in our own laboratory, with our own instrumentation. However, the initial data imply that it will not be necessary to microdissect or otherwise fractionate DCIS specimens prior to TC analysis.

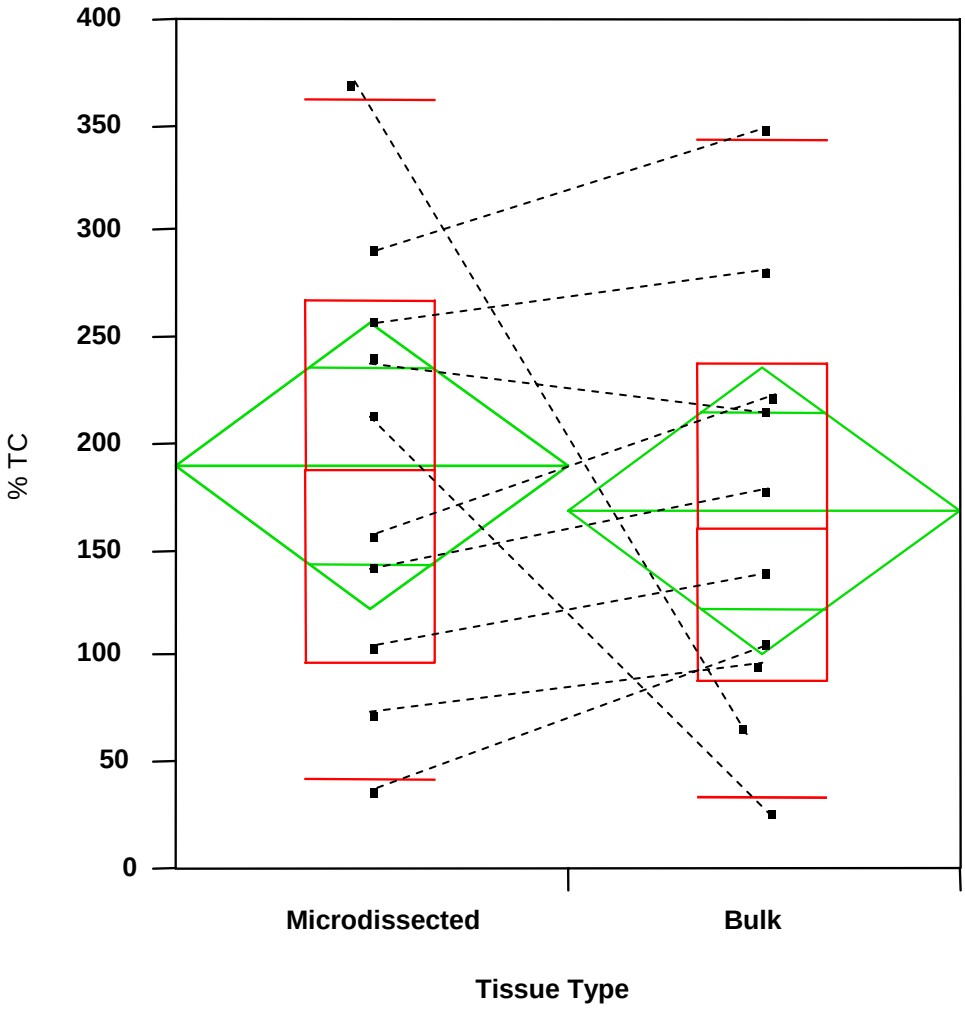

Figure 2. Distributions of Telomere DNA Contents (TC) in Ten Pairs of Microdissected and Bulk DCIS Tissues. TC is shown on the y-axis, and is expressed as a percentage of TC in placental DNA standard, measured in parallel. The line across the middle of each box shows the group median and the quartiles (25th and 75th percentiles) as its ends. The 10th and 90th quantiles are shown as lines above and below the box. The line across each diamond represents the group mean. The height of each diamond represents the 95% confidence interval for each group.

Tasks 5 and 6 have been completed.

Tasks 7 and 8 are ongoing. We have obtained 97 samples of DCIS tissue from women with 4-9 years of continuing follow up, have purified DNA, measured TC, and compared these data to TC measured in invasive breast tumors (Figure 3). Non-parametric Rank Sums (Kruskal-Wallis) test demonstrates a statistically significant difference between the mean TC in DCIS tissue and either invasive or metastatic cancers ($p<0.0001$). The New Mexico Tumor Registry is currently working to identify another 120 specimens of DCIS from women with longer follow up, including as many as possible who had recurrent disease.

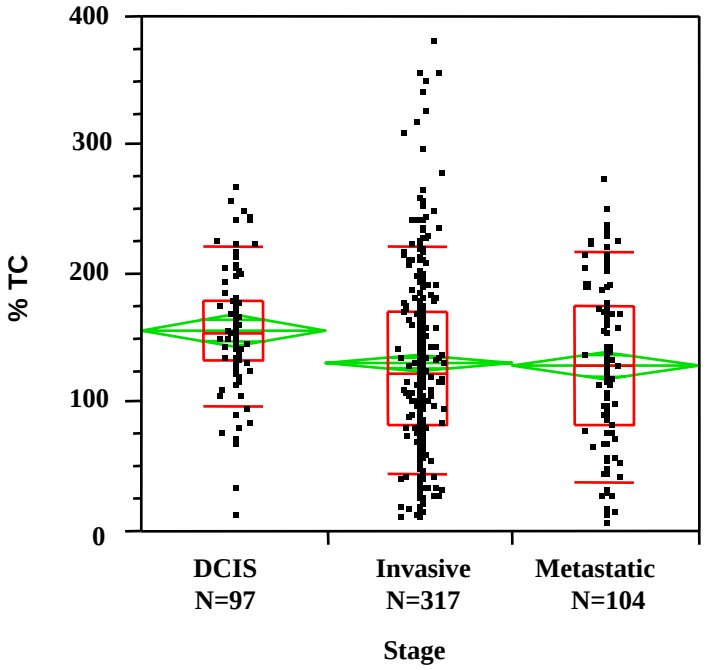

Figure 3. Distributions of Telomere DNA Contents (TC) in DCIS, Invasive and Metastatic Breast Tumors. TC is shown on the y-axis, and is expressed as a percentage of TC in placental DNA standard, measured in parallel. The number of specimens in each tissue set (N) is indicated below the set designation on the x-axis. The line across the middle of each box shows the group median and the quartiles (25th and 75th percentiles) as its ends. The 10th and 90th quantiles are shown as lines above and below the box. The line across each diamond represents the group mean. The height of each diamond represents the 95% confidence interval for each group.

KEY RESEARCH ACCOMPLISHMENTS

- Laser capture microscopy was used to microdissect 10 specimens of DCIS tissues.
- Telomere DNA content has been analyzed in 10 pairs of bulk and microdissected DCIS tissues.
- Telomere DNA content has been analyzed in 97 specimens of bulk DCIS tissues.
- Search parameters for the subject cohort for the future retrospective investigation have been designed and submitted to the New Mexico Tumor Registry.
- Tissue procurement has been initiated.

REPORTABLE OUTCOMES

- A database has been produced that contains anonymous patient histories, including age at diagnosis, ethnicity, treatments, tumor stage, estrogen and progesterone receptor status, tumor size, length of disease free survival or date and cause of death and diagnosis.
- DNA banks from DCIS, Invasive and metastatic breast tumor tissues have been produced.
- Initial data from this investigation (contained in Figures 1 and 3) is included in one paper that is in press and a second manuscript that is in preparation.

CONCLUSIONS

- Prior studies show that telomere DNA content (TC) is a novel and independent prognostic marker in breast tumors. The data obtained thus far indicate that meaningful TC measurements can be obtained with bulk DCIS tissues and that TC is associated with tumor stage. This suggests that TC in DCIS tissue may have similar prognostic value.

REFERENCES

1. Ernster VL, Barclay J, Kerlikowske K, Grady D, Henderson C. Incidence of and treatment for ductal carcinoma in situ of the breast. JAMA, 275:913-8, 1996.

2. Breast Cancer Facts and Figures: 2003-2004. American Cancer Society, Atlanta, GA, 2004.

3. Burstein, HJ, Polyak, K, Wong, JS, Lester, SC and Kaelin, CM. Ductal Carcinoma in Situ of the Breast. N Engl J Med 350:1430-41, 2004.

4. Page DL, Dupont WD, Rogers LW, Jensen RA, Schuyler PA. Continued local recurrence of carcinoma 15- 25 years after a diagnosis of low grade ductal carcinoma in situ of the breast treated only by biopsy. Cancer 76:1197-200, 1995.

5. Silverstein MJ, Barth A, Poller DN, Gierson ED, Colburn WJ, Waisman JR, Gamagami P. Ten-year results comparing mastectomy to excision and radiation therapy for ductal carcinoma in situ of the breast. Eur J Cancer 31A:1425-7, 1995.

6. Silverstein MJ, Poller DN, Barth A, Waisman JR, Jensen JA, Masetti R, Gierson ED, Colburn WJ, Lewinsky BS, Auerbach SL, Gamagami P. Intraductal breast carcinoma: experiences from the Breast Center in Van Nuys, California. Recent Results Cancer Res 140:139-53, 1996.

7. Graversen HP, Blichert-Toft M, Dyreborg U, Andersen J. In situ carcinomas of the female breast. Incidence, clinical findings and DBCG proposals for management. Acta Oncol 27:679-82, 1988.

8. Betsill WL Jr, Rosen PP, Lieberman PH, Robbins GF. Intraductal carcinoma: longterm follow-up after treatment by biopsy alone. JAMA 239:1863-7, 1978.

9. Eusebi V, Feudale E, Foschini MP, et al. Long-term follow-up of in situ carcinoma of the breast. Semin Diagn Pathol. 11:223-35, 1994

10. Ernster VL, Barclay J, Kerlikowske K, Wilkie, H, Ballard-Barbash, R. Mortality among women with ductal carcinoma in situ of the breast in the population-based Surveillance, Epidemiology and End Results Program. Arch. Intern. Med. 160:953-958, 2000.

11. Baxter NN, Virnig BA, Durham SB, Tuttle TM. Trends in the treatment of ductal carcinoma *in situ* of the breast. J. Nat'l Cancer Inst. 96:443-448, 2004.

12. Morrow M. The certainties and the uncertainties of ductal carcinoma in situ. J. Nat'l Cancer Inst. 96:424-425, 2004.

13. Fordyce CA, Heaphy CM, Griffith JK. Chemiluminescent measurement of telomere DNA content in biopsies. Biotechniques 33: 144-8, 2002.

14. Bryant JE, Hutchings KG, Moyzis RK, Griffith JK. Measurement of telomeric DNA content in human tissues. Biotechniques, 23:476-8, 1997.

15. Griffith JK, Bryant JE, Fordyce C, Gilliland F, Joste NE, Moyzis RK.Reduced telomere DNA content is correlated with genomic instability and metastasis in invasive human breast carcinoma. Breast Cancer Research & Treatment, 54, 59-64, 1999.

16. Donaldson, L., Fordyce, Gilliland F, Smith AY, Feddersen R, Joste NE, Moyzis RK, Griffith JK. Association between outcome and telomere DNA content in prostate cancer. J. Urology. 162: 1788-92, 1999.

17. Fordyce CA, Heaphy CM, Joste NE, Smith AY, Hunt WC, and Griffith JK. Association Between Cancer-free Survival and Telomere DNA Content in Prostate Tumors. J. Urology, 173: 610-614, 2005.

18. Fordyce CA, Heaphy CM, Joste NE, Bisoffi M, Wyaco JL, Joste NE, Mangalik A, Baumgartner KB, Baumgartner RN, Hunt WC, and Griffith JK. Telomere Content Correlates with Stage and Prognosis in Invasive Breast Cancer, Breast Cancer Research and Treatment, in press.